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GENERATION AND CHARACTERIZATION OF CHROMOSOME 2-SPECIFIC COSMID, FOSMID AND PAC CLONE LIBRARIES AND NEW CHROMOSOME 2 STSs DERIVED FROM MICRO-DISSECTION CLONES

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The National Laboratory Gene Library Project has been constructing chromosome-specific clone libraries for a number of years. Chromosome 2-specific cosmid and fosmid libraries have recently been constructed from flow-sorted chromosome 2 from the hybrid cell line GM10826. The cosmid library consists of ~45,000 arrayed clones (~4.5 X coverage) and the fosmid library consists of ~27,000 arrayed clones (~2.5 X coverage). Approximately 65% of the clones in both libraries are derived from chromosome 2 and have an average insert size of 40 kb. DNA pools from both libraries have been made and PCR screening has been used to confirm the extent of coverage of individual STS markers on chromosome 2 within the libraries and to isolate clones containing genes of interest.

A chromosome 2 PAC library has also been generated from the hybrid cell line GM10826. Ligations containing clones greater than 80 kb average size are being plated and the chromosome 2 clones identified by colony hybridization. To date, approximately 4,500 chromosome 2 clones, ~ 1.5 X chromosome 2 coverage, have been arrayed into microtiter plates. Additional clones are continuing to be arrayed. The chromosome 2 representation of the library is also being tested by PCR using STS primers distributed along the chromosome and to identify clones containing genes of interest on chromosome 2.

Chromosome 2-derived microdissection clones obtained from F.-T. Kao at the University of Colorado are being used to generate new regionally assigned STSs. Microclones are sequenced and the sequences used to generate new STSs. The new STSs have been localized by PCR to YACs which have been tied to the genetic map of the chromosome by CEPH. The new STSs have also been localized to the cytogenetic map of the chromosome by identifying both PAC and cosmid clones associated with the STSs and FISH mapping these clones. Combining the chromosome 2-specific libraries along with the new STSs will allow for the construction of a high quality, high resolution physical map of the chromosome and will provide important resources for sequencing of the chromosome.

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Poster

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